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Christine Todd Whitman, Administrator
US Environmental Protection Agency
Ariel Rios Building
Room 3000, #1101-A
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: Comments on the HPV test plan for 2-H-Benzimadazole-2-thione, 1, 3-dihydro-4 (or 5)-methyl-, Zinc Salt (2:1)

Dear Administrator Whitman,

The following are comments on the test plan for Zinc Mercaptotolumidazole (ZMTI), prepared by The R. T. Vanderbilt Company, Inc. (Vanderbilt). These comments are submitted on behalf of the Physicians Committee for Responsible Medicine (PCRM), People for the Ethical Treatment of Animals (PETA), the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These animal, health, and environmental protection organizations have a combined membership of more than ten million Americans.

We would like to comment specifically on the acute fish aquatic toxicity test (OECD Test Protocol 203) proposed by Vanderbilt, which will result in the death of at least 40-120 animals. We disagree that this test needs to be performed, for the following reasons:

1. *The low water solubility of ZMTI*

   Given the low acute toxicity of ZMTI in rats (LD_{50} = 800 mg/kg), it is unlikely that the chemical would dissolve in water at a sufficiently high dose to show an effect during an acute fish test. Vanderbilt describes ZMTI as having "negligible water solubility" (Test Plan p. 1), and reports in the Robust Summary that only 32 mg/l will dissolve. Again, given information already known, it is questionable that a toxic endpoint will be reached.

2. In vitro *and* in silico *methods are available*

   As in our comments on more than 30 previous test plans in the HPV program, we urge Vanderbilt to use alternatives to the acute fish toxicity test, such as ECOSAR, TETRATOX, or the recently validated *Dar*T test (see Appendix).

3. The ecologic relevance of fish toxicity should be taken into consideration

The purpose of fish tests is not for predicting toxicity in individual fish, but for predicting economic loss (to commercial and "sport" fisheries) and ecologic damage (fish are an important part of the food chain). The test therefore aims to show whether pollution with ZMTI will result in large-scale fish death. However, water pollution can wipe out fish stocks even with no direct toxicity, because killing the food of the fish will lead to starvation. Carps and catfishes are herbivorous, eating mostly algae, whereas most other familiar North American freshwater fish species are carnivorous, eating worms, small crustaceans, smaller fish, insect larvae, etc. However, the toxicity of ZMTI towards these types of organism is unknown, as shown in the Test Plan. Fish tests should not be carried out while other types of aquatic toxicity are unknown.

According to the October 1999 agreement letter sent to the High Production Volume Chemical Challenge Program participants, "animal experiments should not be performed if another validated method—not involving the use of animals—is reasonably and practically available." Clearly, Vanderbilt could substitute any one of the above methods for the proposed OECD Test Protocol and prevent the suffering and death of at least 40-120 fish.

We appreciate the opportunity to comment on this test plan, and I look forward to a prompt and favorable response to our concerns. I may be reached at 202-686-2210, ext. 335, or via email at *kstoick@pcrm.org*.

Sincerely,

Kristie Stoick, MPH
Research Analyst

Chad Sandusky, PhD
Director of Research

Appendix: *In vitro* and *in silico* fish toxicity test methods

TETRATOX, an assay based on the protozoan *Tetrahymena pyriformis* (Larsen 1997), is an appropriate method for use in this plan. With 50% growth impairment as the endpoint, the results of this assay show close similarity to toxicity in the fathead minnow (Schultz 1997). The extensive available information demonstrates that TETRATOX is an effective alternative to fish testing. It is in fact already used extensively in industry, and is being considered for regulatory acceptance by the OECD. It is also rapid, easy to use, and inexpensive.

The recently validated *Dar*T test is another prospective replacement for *in vivo* tests. The test protocol and performance parameters are given in detail in Schulte (1994) and Nagel (1998). Briefly, however, the *Dar*T test uses fertilized zebrafish (*Danio rerio*) eggs as a surrogate for living fish. The exposure period is 48 hours, and endpoints assessed include coagulation, blastula development, gastrulation, termination of gastrulation, development of somites, movement, tail extension, eye development, circulation, heart rate, pigmentation and edema. Endpoints comparable to *in vivo* lethality include failure to complete gastrulation after 12 hours, absence of somites after 16 hours, absence of heartbeat after 48 hours, and coagulated eggs. The other endpoints provide further insight for a more detailed assessment of test substances. The reliability and relevance of the *Dar*T test have recently been confirmed in an international validation study coordinated and financed by the German Environmental Protection Agency; predictions of acute toxicity from the *Dar*T test were highly concordant with *in vivo* reference data (Schulte 1996). This *in vitro* test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius 1995), and is clearly suitable for immediate use as a replacement for the use of fish in the HPV program's screening-level toxicity studies.

With respect to *in silico* methods, several quantitative structure-activity relationship (QSAR) programs for estimating toxicity to fish and other aquatic organisms are available. The EPA itself encourages the use of one established QSAR: ECOSAR (See http://www.epa.gov/oppt/newchems/21ecosar.htm; EPA 2002a).

References

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